A synthesized pheromone induces upstream movement in female sea lamprey and summons them into traps

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Female insect pheromone blends induce robust tracking responses in males and direct them into traps. In vertebrates, pheromones that induce strong and precise tracking responses in natural habitats have rarely been described. Here, we show in the sea lamprey (*Petromyzon marinus*), a vertebrate invader of the Laurential Great Lakes, that a synthesized component of the male mating pheromone, 7α, 12α, 24-trihydroxy-5α-cholan-3-one 24-sulfate (3kPZS), when released into a stream to reach concentrations of 10^{-14}, 10^{-13}, 10^{-12} ,10^{-11}, or 10^{-10} M, triggers robust upstream movement in ovulated females drawing ≈50% into baited traps. Experiments conducted in diverse stream segments demonstrate the level of behavioral response was not affected by habitat conditions and is effective over hundreds of meters. 3kPZS is equally effective at luring ovulated females as the whole pheromone blend released by males between 10^{-14} and 10^{-11} M. 3kPZS diverts ovulated females away from and disrupts orientation to male washings when applied at concentrations higher than washings. Indeed, a single pheromone compound is able to redirect female sea lampreys away from a natural pheromone source and lure them into traps, which should be more effective than targeting males when applied in population control. Our findings may spur the discovery of other potent and environmentally benign agents to combat biological invasion, a process accelerated by globalization, exacerbated by climate change, and costing the global economy US$ 1.4 trillion of damage annually.

invasive species | odorant tracking | pest control | odorant disruption | jawless vertebrate

Pheromones are naturally occurring chemical signals and thus environmentally benign agents for pest control. In insect species, blends of synthesized female pheromones have been used for decades to lure males into traps (1) and disrupt reproduction (2). Vertebrate pests have never been controlled with pheromones. Studies of pheromone-mediated behavior of vertebrates in natural habitats have been limited both because relatively few vertebrate pheromones have been chemically identified (3) and because field research on this topic is often constrained by the ecology and behavior of vertebrates (4). Nonetheless, studies of the sea lamprey (*Petromyzon marinus*), an ancestral vertebrate and destructive invader of the Laurentian Great Lakes (5), indicate that spermiated males release a pheromone, 7α, 12α, 24-trihydroxy-5α-cholan-3-one 24-sulfate (3kPZS) (6), that induces predictable movements in ovulated females in spawning streams (7). We reasoned that the sea lamprey offers a uniquely advantageous model for determining possible applications of pheromones in vertebrate pest control.

Odors, both in air and water, occur as turbulent plumes in which intermittent discrete packets and filaments of odorants at high and intermediate concentrations are interspersed with regions of below threshold concentrations (8, 9). These plumes are typically mixtures containing more than a single biologically relevant compound (10). Temporal information present in odor plumes appears to be used by moths (8) and crustaceans (11) to assess the direction and perhaps the distance of the odor source.

For the sea lamprey, odor plumes of 3kPZS released by mature males also challenge ovulated females with a comparable level of temporal and spatial complexity. Lamprey spawning riffles are often separated over kilometers by stream segments that have highly variable fluid dynamics. An orientation strategy that relies on concentration gradients (chemotaxis) may not be effective for ovulated females to track a 3kPZS plume because sea lampreys are monorhinic and move scented water into and out of the olfactory capsule through a single nostril with each respiratory cycle (12). This limits odor plume sampling to a few "sniffs" per second and restricts ovulated females to sequential sampling of odor plumes (klinotaxis). This sampling rate appears to be too slow to obtain reliable measurements of the dynamic properties in turbulent odor plumes (9) to permit chemotaxis. We hypothesized that ovulated females could move close to a source of 3kPZS by simply swimming upstream when the odorant is detected and searching when it is lost, that is, chemically mediated upstream movement.

If this is true, 3kPZS may be highly effective at luring ovulated females into specific stream locations. Therefore, our main objective in studying the behavioral response of ovulated females to 3kPZS is to assess the potential utility of 3kPZS in control of sea lamprey in the Laurentian Great Lakes (5). Over 3,000 insect pheromones have been identified (13) and mixtures of female pheromones are commonly used as agents to enhance male trapping efficiency and disrupt reproduction as benign alternatives to traditional pesticide treatments (14). 3kPZS offers an ideal model for developing pheromone-based vertebrate pest control because, unlike mixtures of insect pheromones that attract males, 3kPZS alone modifies the behavior of ovulated females in their natural habitat (7). Further, 3kPZS is the first synthesized vertebrate pheromone in which an experimental user permit from US Environmental Protection Agency has been issued to allow application in a natural stream. The overall goal of the study was to describe the behavioral processes by which ovulated females locate 3kPZS to reveal the efficacy of 3kPZS as a control agent.

Results

Synthesized 3kPZS Induces Upstream Movement in Ovulated Females and Lures Them into Traps. Ovulated females in spawning streams encounter variable fluid dynamics and spermiated male populations (15). Therefore, we postulated that ovulated females

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Table 1. 3kPZS-induced upstream movement directs females into traps

Treatment		Distribution			Orientation			
Trap Bait, M	*n*	Upstream, *n*	Captured, n	Statistics	Time, min	Rests	Downstream	Sidestream
3kPZS 10^{-11}	25	19 A	11 *AB*		17.7 (21.9) A	3.9 (5.1) A	0.4 (0.8) A	1.8 (2.6) A
3kPZS 10^{-12}	25	18 A	14 *A*		20.8 (24.0) A	4.9 (5.3) A	0.0 (0.0) A	0.5 (0.7) A
3kPZS 10^{-13}	24	13 AB	9 *AB*		11.3 (9.3) A	2.8 (2.2) A	0.2 (0.4) A	2.0 (2.9) A
3kPZS 10^{-14}	24	8 B	6 *BC*		NA	NA	NA	NA
Control	22	11 AB	1 *C*		NA	NA	NA	NA
	X^2	12.29	18.64	F-value	0.38	1.15	0.01	1.12
	df	4	4	$^{NDF}/_{DDF}$	2/27	2/23	2/23	2/23
	p-value	0.015	0.001	p-value	0.689	0.334	0.99	0.345

Distribution is the number of ovulated females that moved upstream and were captured when one trap was baited with control solvent and the other trap was baited with 3kPZS, and when both traps were baited with control solvent (Control). All ovulated females were captured in 3kPZS-baited traps when 3kPZS was applied to the stream. Orientation summarizes behaviors of ovulated females that were captured in 3kPZS-baited traps: Time, mean (SD) time to swim from the release cage and enter trap; Rests, mean (SD) number of rests; Downstream, mean (SD) number of downstream movements; and Sidestream, mean (SD) sidestream movements taken by each ovulated female while moving upstream toward the 3kPZS-baited trap. Distribution data were evaluated with logistic regression and orientation behaviors evaluated with general linear models. 3kPZS treatments that share a letter were not significantly different ($\alpha = 0.05$). Orientation statistics were not evaluated for control and 3kPZS 10^{-14} M treatments.

would be able to locate sources of 3kPZS that vary in concentration, and subsequently, be guided into 3kPZS-baited traps. To test this prediction, we observed whether ovulated females were able to locate the exact source of 3kPZS by baiting traps with 3kPZS, recording ovulated female capture rates and tracking ovulated female movements, thereby also revealing the utility of 3kPZS as a sea lamprey-control agent. Experiments were conducted in a natural spawning stream divided into two channels by an island (Ocqueoc River, MI) (16). A sea lamprey trap was placed in each channel and one trap randomly received 3kPZS to achieve a concentration of 10^{-11}, 10^{-12}, 10^{-13}, or 10^{-14} M and the other trap received methanol as a control for the solvent in which the pheromone was dispersed in the treatment traps (Table 1 and supporting information (SI) Fig. S1). Concentration was calculated assuming complete mixing with stream discharge, which was confirmed to occur 70 m downstream by dye tests. We released ovulated females 70 m downstream and found that traps baited with 3kPZS 10^{-11}, 10^{-12}, and 10^{-13} M did not differ in capture rate (logistic regression; $X^2 = 1.75$, df = 2, $P = 0.417$) and captured 46% of ovulated females released and 68% of ovulated females that moved upstream (Movie S1). Even at 10^{-14} M, 3kPZS-baited traps captured 25% of ovulated females released and 75% of ovulated females that moved upstream, which was more than the control trap (binomial distribution, $P = 0.012$).

Behavioral observations show that ovulated females oriented toward 3kPZS-baited traps by swimming directly upstream (Fig. 1). Ovulated females captured in traps baited with 3kPZS at 10^{-11}, 10^{-12}, or 10^{-13} M did not differ in time taken to swim upstream into the trap after leaving the release cage (mean = 18.1 min, range 2.7 to 84.8 min), the number of rests (mean = 3.8, range 0 to 16), number of downstream movements (mean = 0.2, range = 0 to 2), or number of sidestream movements (mean = 1.3, range = 0 to 7) (Table 1). Only 17% of ovulated females exhibited two or more sidestream movements while moving upstream toward the 3kPZS-baited trap. Ovulated females located the exact release point of 3kPZS even when concentrations varied 1,000-fold.

We further reasoned that ovulated females would not become adapted to 3kPZS even after prolonged exposure in 3kPZS plumes during their directed upstream movement over long distances and in diverse river habitats. We tested this hypothesis by recording ovulated female responses to 3kPZS over a 650-m distance at two experimental sites in the Ocqueoc River. One segment was located on the sea lamprey spawning riffle used in previous experiments and the other site was located several km downstream of the spawning riffle that was characterized by slow deep flow and sandy bottom (run) (Fig. S2). At each experimental site, we released ovulated females 650 m downstream of a trap baited with 3kPZS to reach 10^{-12} M and a trap baited with control solvent.

3kPZS induced directed upstream movement >650 m in both environments. In the riffle and run stream segments, the proportion of ovulated females moving upstream and entering within 1 m of 3kPZS-baited traps did not differ, showing that 3kPZS induced equally strong migrations in both habitats (Fisher's exact; $P = 0.738$). More ovulated females moved upstream and entered within 1 m of the baited traps when 3kPZS was applied to the stream than when control solvent was applied to both traps (Table S1; Fisher's exact; riffle: $P < 0.001$; run: $P = 0.005$). However, the proportion of ovulated females captured in 3kPZS-baited traps was greater in the riffle habitat than in the run habitat (Fisher's exact; $P = 0.002$) and the time for ovulated females to enter within 1 m of the 3kPZS-baited trap was longer in the riffle stream segment than the run segment (Wilcoxon rank-sum test; $P = 0.027$, $z = -2.21$, df = 30). Slower swimming speeds of ovulated females in the riffle may be due to fast water velocity and the inefficiency of anguilliform swimming. Similarly, high water velocity through the trap at the riffle site may have caused ovulated females to swim with great effort into the trap funnel resulting in higher capture efficiency. 3kPZS elicits long upstream migration in both environments, but the trapping techniques used in this study may be most efficient if traps are placed in riffle environments.

Role of 3kPZS as a Component of the Pheromone Mixture. Given that most characterized pheromones are mixtures that only elicit strong responses when all components are present (17), it was interesting to observe that 3kPZS alone induced robust upstream movements over long distances and ranges of concentrations. It has been hypothesized that spermiated male washings (SMW) contain additional pheromone components (18) that induce near source search behaviors in ovulated females (7). Thus, we wished to confirm the role of 3kPZS-mediated upstream movement when placed in the context of SMW by directly comparing responses of ovulated females to synthesized 3kPZS and SMW over long and short distances. SMW were used instead of live spermiated males to provide an unequivocal test of whether

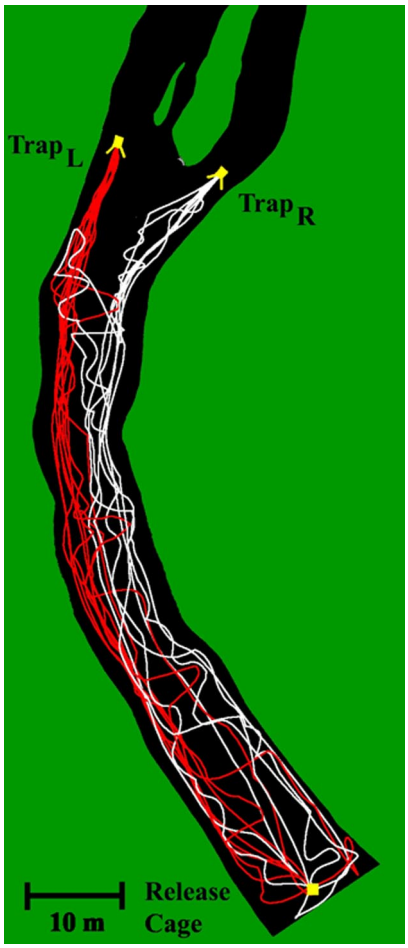

Fig. 1. 3kPZS-baited traps capture all females when compared with unbaited traps. Observed movements of individual ovulated females trapped when 3kPZS was applied at 10^{-11} M, 10^{-12} M, or 10^{-13} M in a randomly selected trap and when control solvent was applied in the other ($Trap_L$ and $Trap_R$). Red lines illustrate ovulated females entering the left trap when the left trap was baited with 3kPZS. White lines illustrate ovulated females entering the right trap when the right trap was baited with 3kPZS. Green illustrates ground and black illustrates river.

additional pheromone components induce near source search behaviors. Previously, we found behavioral responses of ovulated females to spermiated males or their washings in a two-choice maze do not differ (7). In a natural stream, traps baited with spermiated males and SMW both capture large proportions of ovulated females (16, 19). These results are not surprising because ovulated females are blind (15) and naris-plugged ovulated females are not able to locate spermiated males over long or short distances (19). Therefore, by comparing 3kPZS and SMW, we also evaluated the potential utility of 3kPZS in redirecting ovulated females away from natural sources of pheromone, and thus a potential mate.

At the spawning riffle segment, we constructed a lamprey nest in each river channel 45 m upstream of the confluence of the channels (Fig. S3*a*). In one nest we applied SMW to reach an in-stream natural 3kPZS concentration of 7.5×10^{-13} M. In the other nest (other channel), synthesized 3kPZS was applied at 0.7, 1.0, 1.3, or 3.3 times the concentration of natural 3kPZS in SMW. Females were released 250 m downstream and had to choose which channel to enter 45 m downstream of the odor sources. Surprisingly, when applied at equal 3kPZS concentrations, synthesized 3kPZS and SMW attracted equal proportions of ovulated females, and at merely 3.3 times the concentration of 3kPZS in SMW, synthesized 3kPZS attracted 84% of responsive ovulated females (Table 2). Notably, nest observations show that ovulated females spent 10-fold more time in nests baited with SMW than nests baited with 3kPZS (Table 2 and Movie S2).

The distribution of ovulated females between channels baited with 3kPZS and SMW show that 3kPZS plays a major role in inducing directed upstream movement, but a higher potency of SMW in retaining ovulated females on nests suggests that additional components increase near source retention. To confirm this finding, we compared the near source effects of 3kPZS and SMW by building two spawning nests 1.25 m apart (Fig. S3*b*) and applying SMW to one nest to reach a natural 3kPZS concentration of 7.5×10^{-13} M and synthesized 3kPZS to the other at 1.3 or 3.3 times the concentration of natural 3kPZS in SMW. Contrary to results from 45-m comparison experiments, all ovulated females went to the SMW when synthesized 3kPZS was applied at 1.3 times, and equal proportions of ovulated females visited both nests when 3kPZS was applied at 3.3 times (Table 2). Again, SMW retained ovulated females ≈10 times longer than synthesized 3kPZS.

Why did SMW and 3kPZS equally attract ovulated females to nests over a 45-m distance when applied at 7.5×10^{-13} M, but synthesized 3kPZS did not retain females on nests? It is possible that, at this 3kPZS concentration, the pheromone components within SMW that attract and retain ovulated females near nests may not be detected long distances downstream due to lower release rates or olfactory sensitivities (or both). To investigate

Table 2. Female preference for synthesized 3kPZS and natural pheromone mixture

Treatment		Distribution			Retention		
Direct comparison	*n*	3kPZS, *n*	SMW, *n*	*P* value (df;X^2)	3kPZS, sec	SMW, sec	*P* value (NDF/DDF;F-Stat)
45 m: 0.7 × 3kPZS vs. SMW	38	1	24	<0.001 (1; 20.81)	60	1849	0.051 (1/23; 4.27)
45 m: 1.0 × 3kPZS vs. SMW	41	9	15	0.078 (1; 3.11)	152	618	0.010 (1/21; 8.00)
45 m: 1.3 × 3kPZS vs. SMW	36	12	8	0.315 (1; 1.01)	8	447	0.001 (1/18; 14.29)
45 m: 3.3 × 3kPZS vs. SMW	36	16	3	0.035 (1; 4.45)	80	419	0.015 (2/16; 5.52)
0 m: 1.3 × 3kPZS vs. SMW	21	0	19	<0.001 (1; 29.06)	NA	439	NA
0 m: 3.3 × 3kPZS vs. SMW	40	12	13	0.583 (1; 0.30)	30	342	<0.001 (1/23; 19.11)
45 m: 10^{-11} M 3kPZS vs. SMW	60	18	10	0.184 (1; 1.76)	123	530	0.035 (1/26; 4.94)
45 m: 10^{-12} M 3kPZS vs. SMW	38	11	10	0.247 (1; 1.34)	197	874	<0.001 (1/19; 25.33)
45 m: 10^{-13} M 3kPZS vs. SMW	60	11	14	0.488 (1; 0.48)	208	297	0.097 (1/23; 3.00)
45 m: 10^{-14} M 3kPZS vs. SMW	83	9	11	0.276 (1; 1.19)	70	64	0.717 (1/18; 0.19)

Distribution of ovulated females when presented with nests baited with synthesized 3kPZS or spermiated male washings (SMW; natural pheromone) and median time spent within 0.5 m of the nest (Retention). Data from two experimental designs are presented; when ovulated females choose an odor source from 45 m downstream (Treatment: 45 m; Fig. S3*a*) and when the odorants were 1.25 m apart (Treatment: 0 m; Fig. S3*b*). Distribution data were evaluated with logistic regression and retention data were evaluated with a general linear model. NA, data not applicable.

this possible scenario, we directly compared 3kPZS and SMW at a 45-m distance across a 1,000-fold change in concentration. Responses to SMW and 3kPZS were directly compared when the 3kPZS concentration of both sources were equal to 10^{-11}, 10^{-12}, 10^{-13}, and 10^{-14} M, respectively. At each concentration, 3kPZS and SMW triggered equal proportions of ovulated females to move upstream into the baited channels (Table 2), showing that within the range of concentrations tested, 3kPZS is the only pheromone component that influences long distance responses in ovulated females. As expected, retention in the SMW nest was significantly greater than synthesized 3kPZS at 10^{-11} M and 10^{-12} M. However, retention in the SMW nest and 3kPZS nest did not differ at 10^{-13} M and 10^{-14} M, perhaps because at extremely low concentrations minor components were not detectable even when ovulated females were on the nest.

3kPZS Disrupts Female Orientation to Male Pheromone. Given the dominant role 3kPZS plays in the pheromone mixture to induce upstream movement over various distances, we postulated that high concentrations of 3kPZS can disrupt both near and far source effects of the natural male pheromone blend. We conducted an experiment to test this hypothesis and further confirm that 3kPZS indeed maintains robust movement directly upstream over a wide range of concentrations. At the spawning riffle, we applied SMW 20 m upstream of the ovulated female release site to reach an in-stream natural 3kPZS concentration of 10^{-12} M and a background synthesized 3kPZS source was applied 40 m upstream of the SMW source so that the synthesized 3kPZS-plume enshrouded the SMW-plume (Fig. 2). Background concentrations of 3kPZS applied were 0 (vehicle solution), 10^{-12}, 10^{-11}, or 10^{-10} M.

Consistent with our hypothesis, a higher proportion of ovulated females completely missed the enshrouded SMW source while swimming upstream to the 10^{-10} M synthesized 3kPZS source than to the control source (Table 3 and herein; logistic regression; $X^2 = 4.24$, df = 1, $P = 0.040$). To discern the mechanism for this disruptive effect, we compared individual ovulated female movement tracks to the plume distribution as determined by dye tracing (Fig. 2). When 10^{-10} M 3kPZS was applied, ovulated females were more widely distributed across the stream and were less likely to track the highest concentration of natural 3kPZS originating from SMW (Figs. 2 and S4 and Table S2; ANOVA; F = 5.795, df = 3/20, $P = 0.005$). Furthermore, a higher proportion of ovulated females swam upstream of the SMW source when 3kPZS was applied at 10^{-12}, 10^{-11}, or 10^{-10} M than when control solvent was applied. For those ovulated females that did visit the SMW source, they spent less time within 0.5 m of the SMW release point when 10^{-11} M or 10^{-10} M synthesized 3kPZS was applied upstream (Student's *t* test; *t*-value = −2.81, df = 81, $P = 0.063$ and t-value = −2.61, df = 81, $P = 0.011$, respectively). When ovulated females moved upstream of the background source of synthesized 3kPZS they exhibited more sidestream and downstream movements (Table S3).

These experiments also confirm that ovulated females display robust upstream movements to 3kPZS over concentrations ranging 100-fold. First, the proportion of females that moved upstream and located a source of SMW or 3kPZS did not differ when 3kPZS concentration varied from 10^{-12} M to 10^{-10} M (Table 3). Second, swimming speed and swimming distance (Table S4) from SMW to the 3kPZS source did not differ among 3kPZS concentrations. Third, time spent within 0.5 m of the 3kPZS source did not differ among 3kPZS concentrations (Table 3).

Discussion

In natural spawning streams, synthesized 3kPZS applied over a wide range of concentrations lured ovulated females to swim upstream over long distances and subsequently enter traps.

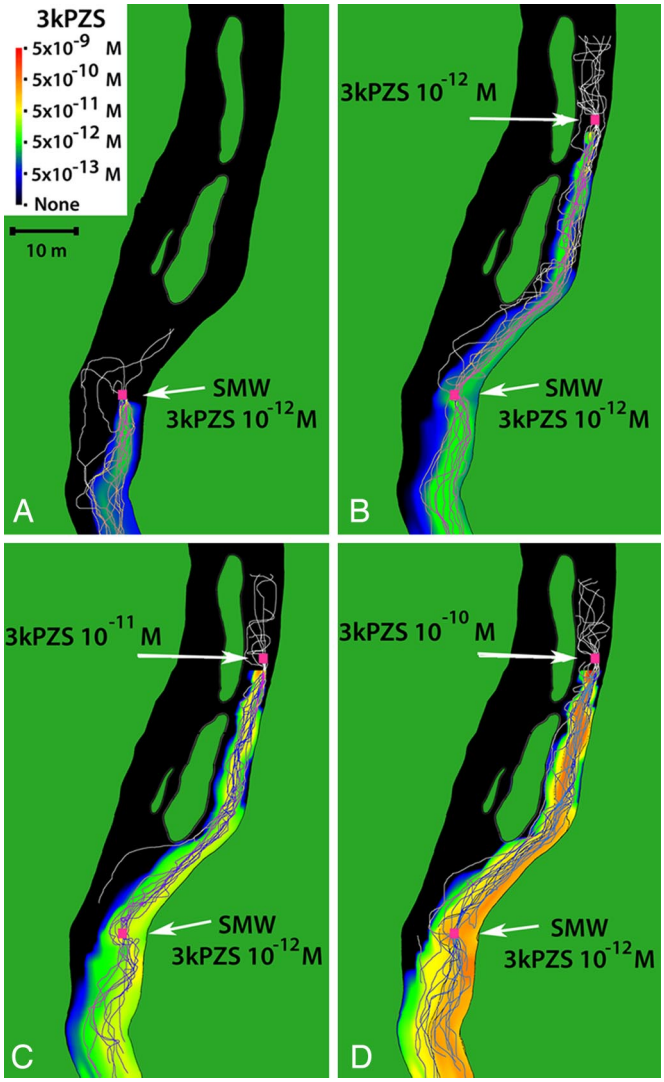

Fig. 2. Female movement tracks during 3kPZS disruption experiments. Synthesized 3kPZS was released 40 m upstream of a source of spermiated male washings (SMW) with natural 3kPZS at 10^{-12} M. Color scale illustrates estimated 3kPZS molar concentrations from both sources of 3kPZS throughout the stream. Background 3kPZS concentrations achieved when fully mixed with the stream discharge. (*A*) No 3kPZS background. (*B*) 3kPZS 10^{-12} M background. (*C*) 3kPZS 10^{-11} M background. (*D*) 3kPZS 10^{-10} M background.

Efficient localization of potential mates is essential for sea lamprey to bring their complex life history to fruition in a single spawning event over a few days before senescence (15). The male sea lamprey mating pheromone facilitates mate finding by signaling to ovulated females the location of spawning grounds and individual nests.

Our data showed that 3kPZS-mediated upstream movement is sufficient and efficient in directing ovulated females to individual nests. A single source of 3kPZS triggered the same directed response in ovulated females over distances of 70 m and 650 m and concentrations varying from 10^{-10} M to 10^{-14} M. It is adaptive for ovulated females to display 3kPZS-induced upstream movement over highly diverse conditions because flow and male abundance differ greatly within and among spawning streams, causing 3kPZS plumes to vary greatly in intensity, and in temporal and spatial profiles. Ovulated females appear to employ the simple orientation strategy of swimming upstream when 3kPZS is detected and moving back downstream and casting side-to-side when the signal is lost.

Table 3. Disruption of female orientation to the natural pheromone enshrouded with 3kPZS

Background 3kPZS	Released, *n*	Responding, *n*	Pass natural, *n*	Visit 3kPZS, *n*	Bypass natural, *n*	Respond, sec	Natural, sec	3kPZS, sec	Speed to 3kPZS, cm/sec
None	37	29 A	6 A	—	1 A	629 A	796 A	—	—
3kPZS 10^{-12}	39	26 A	16 *B*	16 *A*	2 *A B*	1522 A B	820 A B	72 *A*	1.05 A
3kPZS 10^{-11}	39	25 A	20 *B*	20 *A*	4 *A B*	836 A	444 B	178 *A*	0.65 A
3kPZS 10^{-10}	38	28 A	24 *B*	20 *A*	7 *B*	2889 B	358 B	116 *A*	1.06 A
	Test Stat	$X^2 = 2.37$	$X^2 = 19.55$	$X^2 = 1.26$	$X^2 = 7.54$	$F = 3.81$	$F = 3.63$	$F = 2.20$	$F = 2.54$
	df	3	3	2	3	3/99	3/81	2/53	2/52
	p-value	0.499	<0.001	0.534	0.057	0.012	0.016	0.121	0.088

Number of ovulated females released, number entering within 0.5 m of 3kPZS or SMW (Responding), number swimming upstream of SMW (Pass natural), number that entered within 0.5 m of 3kPZS (Visit 3kPZS), and number that entered within 0.5 m of 3kPZS but did not enter within 0.5 m of SMW (Bypass natural). The time for ovulated females to swim 10 m upstream of acclimation cage (Respond), time spent within 0.5 m of natural pheromone (Natural), time spent within 0.5 m of 3kPZS (3kPZS), and swimming speed from SMW source to 3kPZS source. All times are medians. Binary variables evaluated with logistic regression and time variables evaluated with general linear model. Treatments that share a letter are not significantly different (two-tailed $\alpha = 0.05$).

Not only did a vertebrate swim up pheromone plumes, but its efficacy in locating the pheromone sources is comparable with those known in insects. This can be attributed in part to the predictability of shallow river pheromone plumes, which are essentially confined in a one dimensional space by the width, depth, and unidirectional flow of water (20). Moths in a forest environment orient to unpredictable airborne pheromone plumes in a three dimensional space by moving upwind when the pheromone is detected and casting from side to side (optomotor amenotaxis) when the scent is lost (21). Unlike insects, ovulated females oriented toward a single source of 3kPZS by swimming directly into the unidirectional flow (Fig. 1). In the disruption experiments, where an additional source of 3kPZS was located upstream of SMW, ovulated females that bypassed the SMW moved directly upstream to the background source of 3kPZS and subsequently showed more sidestream and downstream movements when ovulated females bypassed the background 3kPZS source (Fig. 2). Ovulated females may use casting as a behavior to ensure that they do not overshoot the spawning grounds and bypass possible mates. It is notable that when ovulated females lose the 3kPZS signal, it takes several seconds (many sniffs) to begin casting, suggesting that either there is a significant integration time to recognize that the signal has been lost, or that upstream movement, once triggered by the pheromone, continues for a period governed by an internal mechanism, as proposed for moths (22). Sea lamprey and moths appear to use similar casting strategies, albeit on different temporal and spatial scales, to relocate lost plumes. A distinct difference is that in a one-dimensional environment, when the odor is briefly lost, it may not be advantageous to immediately move sidestream because plume intermittency may be related to distance from the source rather than location within the stream channel.

In many insects, sex pheromones, like most natural odors, are typically blends of components in specific proportions, with two or more being necessary to elicit a behavioral response (10). In particular, robust long distance behavioral responses are sometimes only elicited when a blend of compounds that function as one signal are present (17). In our experiments, 3kPZS alone elicited robust upstream movements over long distances and was equally effective as the whole pheromone blend found in SMW from 10^{-11} M to 10^{-14} M at attracting ovulated females at a 45 m distance (Table 3). However, males may excrete additional components that function over short distances to retain ovulated females on the nest. In experiments directly comparing 3kPZS and SMW released into nests separated by 1.25 m, SMW retained ovulated females 10-fold more time than 3kPZS (Table 2). Additional evidence that males release additional compounds is gleaned from disruption experiments; when 3kPZS 10^{-10} M was applied, 60% of responding ovulated females located and were retained at source of SMW even when background synthesized 3kPZS 1 m downstream of the SMW source was 5 times greater than the 3kPZS present in the SMW as determined by dye tests (Fig. 2). Recently, several compounds have been isolated from larval sea lamprey washings and subsequently shown to modify behaviors of sexually immature adults in two-choice mazes (23).

Collectively, results show that 3kPZS is a component of the pheromone that functions independently to elicit long distance upstream movements in ovulated females, directing them to nests, and that unidentified components induce near source attraction and retention. The mechanism by which the male sea lamprey mating pheromone coordinates mate finding and reproduction closely resemble the "component" mechanism first described in the pine beauty moth (*Panolis flammea*) (24) and recently described in the red-legged salamander (*Plethodon shemani*) (25) where each component of the pheromone induces separate behaviors such as attraction, landing, or copulation (24). Our data do not support the "blend" hypothesis (17), in which all pheromone components work as one signal to induce all behaviors. However, these hypotheses should be reevaluated when all pheromone components are identified.

From an applied standpoint, our data show that a synthesized pheromone modifies the behavior of ovulated females in their natural habitat and demonstrate the possible utility of 3kPZS as the first synthesized vertebrate pheromone control agent. This hypothesis should be further tested by comparing the effectiveness of 3kPZS versus spermiated males in their natural habitat. Capture rates and effective distances of 3kPZS-baited traps were similar to or greater than those reported in insects (14); but unlike mixtures of insect pheromones that attract males, 3kPZS alone induced robust responses in females. These are distinct advantages of 3kPZS because removal of ovulated females will result in a proportional reduction in viable eggs, and thus be more effective than removal of males. A single compound is less expensive to synthesize, easier to apply, and requires less testing to register with regulatory agencies. In addition to trapping, we show that 3kPZS may be used to divert ovulated females away from natural male pheromones or redistribute ovulated females to tributaries not suitable for spawning or survival of offspring. This approach may be highly effective because sea lamprey only use ≈6% of streams in the Great Lakes basin to spawn (5), and in any particular stream sea lamprey use a small portion of habitat for spawning (15). Furthermore, an "all or nothing" response to 3kPZS over a wide range of concentrations makes control applications even more efficacious because high concentrations are not required to induce strong responses. In the end, 3kPZS-based techniques may provide an environmentally benign means of managing sea lampreys in the Laurentian Great

Lakes, where only 270 g of 3kPZS would be required to activate all currently trapped streams in Lakes Huron, Michigan, and Superior at 10^{-13} M during the 3-week spawning period (5.5 trillion liters of water).

Materials and Methods

Behavior Tests and Permit. Use of sea lampreys was approved under Michigan State University Institutional Animal Use and Care Committee permit 05/06–066-00. Application of 3kPZS and related bioactive components was approved by the Michigan Department of Environmental Quality and United States Environmental Protection Agency through experimental user permit 75437-EUP-2. Experiments were conducted in the Ocqueoc River, MI (7, 16), in stream segments historical infested with larval and spawning sea lamprey (15); however, a barrier several km downstream currently prevents sea lamprey infestation. 3kPZS concentrations were calculated as the final in-stream concentration when completely mixed with the whole stream discharge. Dye tests confirmed that 3kPZS was thoroughly mixed 70 m downstream of the application point. 3kPZS was custom synthesized by Bridge Organics at a purity >95%. A single batch of SMW with a natural 3kPZS concentration of 1.85 mg/L was used in all 3kPZS verse SMW direct comparison experiments in 2007 and a single batch of SMW equaling 3.27 mg/L was used in 2008 direct comparison experiments. A single batch of SMW with natural 3kPZS concentration of 1.5 mg/L was used in all 3kPZS disruption experiments. Ovulated females were fitted with external radio tags (Model 393, Advanced Telemetry System) and tracked using directional radio antenna and receiver (Lotek Engineering Incorporated) (7, 16) during 70 m trapping experiments. In all other experiments, ovulated females were fitted with external passive integrated transponders (PIT tags) and tracked with PIT tag antennas connected to a multiplexer (Oregon RFID). Females were released in groups of three to five for 70-m trapping and in groups of six to 11 for all other experiments. Visual observations of random ovulated females were recorded on stream maps using stream markers as reference points (19). For more information, please see *SI Methods*.

Behavioral Statistics. Female behaviors were assumed to be independent as observed from earlier studies (7, 16). Binary data from experiments with more than two treatment groups were evaluated with logistic regression and models showed no evidence of overdispersion or nonlinearities. Binary data from experiments with two treatment groups were evaluated with a nonparametric Fisher's Exact Test. Time variables and orientation behaviors were evaluated with general linear models where time variables were square-root transformed and orientation behaviors were square-root transformed or ln transformed when needed to meet model assumptions of residual heteroscedasticity and normality. Time data in 650-m trapping experiments were evaluated with a nonparametric Wilcoxon rank-sum test because data could not be transformed to meet parametric statistic assumptions. For 3kPZS versus SWM direct comparison and disruption experiments, data were also analyzed with mixed effect logistic regression and mixed-effect general linear models with a random effect of trial date. All statistical results from general linear models were robust to the inclusion of the random effect of trial date, supporting the assumption that a single ovulated female can be treated as an individual sample (7). Statistical results reported are from two-tailed analyses. A listing of the statistical tests and transformations conducted are in Table S5.

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1. Beroza M, Knipling EF (1972) Gypsy moth control with the sex attractant pheromone. *Science* 177:19–27.
2. Gaston LK, Kaae RS, Shorey HH, Sellers D (1977) Controlling the pink bollworm by disrupting sex pheromone communication between adult moths. *Science* 196:904–905.
3. Brennan PA, Zufall F (2006) Pheromonal communication in vertebrates. *Nature* 444:308–315.
4. Rasmussen LEL, Lee TD, Zhang A, Roelofs WL, Daves GD Jr (1997) Purification, identification, concentration and bioactivity of (Z)-7-dodecen-1-yl acetate: Sex pheromone of the female asian elephant, *Elephas maximus. Chem Senses* 22:417–437.
5. Smith BR, Tibbles JJ (1980) Sea lamprey *Petromyzon marinus* in Lakes Huron, Michigan, and Superior: History of invasion and control, 1936–78. *Can J Fish Aquat Sci* 37:1780–1801.
6. Li W, *et al.* (2002) Bile acid secreted by male sea lamprey that acts as a sex pheromone. *Science* 296:138–141.
7. Siefkes M, Winterstein JS, Li W (2005) 3-keto petromyzonol sulfate specifically attracts ovulating female sea lamprey (*Petromyzon marinus*). *Anim Behav* 70:1037–1045.
8. Murlis J (1997) in *Pheromone Research*, eds Cardé RT, Minks AK (Chapman & Hall, New York), pp 221–231.
9. Webster DR, Weissburg MJ (2001) Chemosensory guidance cues in a turbulent chemical odor plume. *Limnol Oceanogr* 46:1034–1047.
10. Schneider D (1992) 100 years of pheromone research. An essay on Lepidoptera. *Naturwissenschaften* 79:241–250.
11. Grasso FW, Basil JA (2002) How lobsters, crayfishes, and crabs locate sources of odor: Current perspectives and future directions. *Curr Opin Biol* 12:721–727.
12. Kleerekoper H (1972) in *The Biology of Lampreys, Vol* 2, eds Hardisty MW, Potter IC (Academic, New York), pp 373–404.
13. Witzfall P, Lindblom T, Bengtsson M, Toth M (2004) The Pherolist. http://www-.pherolist.slu.se.
14. Howse PE, Stevens ID, Jones OT (1998) *Pest Management* (Chapman & Hall, New York).
15. Applegate VC (1950) *Natural History of the Sea Lamprey in Michigan* (U. S. Department of Interior Fish & Wildlife Service, Washington D.C.).
16. Johnson NS, Siefkes MJ, Li W (2005) Capture of ovulating female sea lampreys in traps baited with spermiating male sea lampreys. *N Am Fish Manage* 25:67–72.
17. Linn CE, Campbell MG, Roelofs WL (1987) Pheromone components and active spaces: What do moths smell and where do they smell it? *Science* 237:650–652.
18. Siefkes MJ, Li W (2004) Electrophysiological evidence for detection and discrimination of pheromonal bile acids by the olfactory epithelium of female sea lampreys (*Petromyzon marinus*). *J Comp Physiol A* 190:193–199.
19. Johnson NS, Luehring MA, Siefkes MJ, Li W (2006) Pheromone induced behavior and pheromone reception in ovulating female sea lampreys. *N Am J Fish Manage* 26:88–96.
20. Zimmer-Faust RK, Finelli CM, Pentcheff ND, Wethey DS (1995) Odor plumes and animal navigation in turbulent water flow: A field study. *Biol Bull* 188:111–116.
21. Kennedy JS, Marsh D (1974) Pheromone-regulated anemotaxis by flying moths. *Science* 184:999–1001.
22. Vickers NJ (2000) Mechanisms of animal navigation in odor plumes. *Biol Bull* 198:203–212.
23. Sorensen PW, *et al.* (2005) Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. *Nat Chem Biol* 1:324–328.
24. Bradshaw JWS, Baker R, Lisk JC (1983) Separate orientation and releaser components in a sex pheromone. *Nature* 304:265–267.
25. Houck LD, *et al.* (2007) A new vertebrate courtship pheromone, PMF, affect female receptivity in a terrestrial salamander. *Anim Behav* 73:315–320.